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Aerial Spray Trials with a Baculovirus to Control Redheaded Pine Sawfly in Ontario in 1979 and 1980.

P. de Groot and J.C. Cunningham

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COVER PHOTO: *Redheaded Pine Sawfly colony feeding on red pine.*

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ABSTRACT

Aerial spray trials were conducted in 1979 to compare the efficacy of a redheaded pine sawfly baculovirus suspension formulated in water, molasses and Sandoz Shade® with a suspension in water alone. In 1980, trials were conducted to compare the efficacy of the same virus dosage emitted at three application rates: 2.35, 4.70 and 9.40 L/ha. Also, in 1980, a comparison was made between the standard, lyophilized, virus-infected insect preparation and a purified virus suspension. The same virus dosage, 5 x 10^9 PIBs/ha, was used in all three tests.

All treatments resulted in complete insect mortality from virus infection. Based on the results of these studies, it is suggested that a crude virus preparation be formulated in water alone and emitted at 5 x 10^9 PIB/ha in a volume of 2.35 L/ha for control of first- and second-instar redheaded pine sawfly larvae. Additional studies on lowest effective application rate and dosage, as well as further replication of the current recommendations, are advocated.

RÉSUMÉ

En 1979, on a réalisé des pulvérisations aériennes afin de comparer l'efficacité d'une préparation d'un baculovirus du diprion de LeConte dans l'eau, la mélasse et le produit Sandoz Shade® à celle d'une préparation dans l'eau seulement. En 1980, des essais ont été réalisés pour comparer l'efficacité de la même dose de virus émise à raison de 2.35, 4.70 et 9,40 L/ha. On a aussi comparé en 1980, la préparation ordinaire d'insectes infectés par le virus et lyophilisés et une suspension purifiée de virus. Dans les trois tests, on a utilisé la même dose virale, 5 x 10^9 corps d'inclusion polyédriques (CIP) à l'hectare.

Tous les traitements ont provoqué par infection virale, une mortalité complète chez les insectes et rendu négligeables les dommages causés au feuillage. D'après les résultats, on propose de préparer une formulation virale brute dans l'eau et de l'épandre à raison de 5 x 10^9 CIP/ha au débit de 2.35 L/ha contre les larves du premier et second stade du diprion. On préconise des études supplémentaires sur les doses et les débits efficaces les plus faibles ainsi que la répétition des recommandations actuelles.

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INTRODUCTION

The redheaded pine sawfly, *Neodiprion lecontei* (Fitch), is a serious defoliator of young hard pine plantations in Ontario and Quebec and to a lesser extent in New Brunswick. Severe defoliation by redheaded pine sawfly can cause reduced height growth, branch mortality, tree deformity, and may lead to tree mortality (Benjamin 1955, MacAloney and Wilson 1964). Tree plantations represent an integral part of forest management and constitute a valuable portion of the forest growing stock. Protection against insects such as redheaded pine sawfly is necessary to ensure that demands for future wood supplies are met.

A nuclear polyhedrosis virus (NPV) from redheaded pine sawfly was discovered in Ontario in 1950 (Bird 1961). Ground spray trials demonstrated that this virus is highly infectious to the sawfly and that a virus epizootic can be initiated which successfully controls this pest (Bird 1961, 1971; Anon 1970).

In 1976, a series of trials was planned to investigate dosages, application equipment, tank mixes and application rates required for an aerial spray program with NPV for control of the redheaded pine sawfly. The results from spray trials conducted in 1976, 1977 and 1978 have been reported previously (Ksupp and Cunningham 1977; Ksupp, *et al.* 1978; de Groot *et al.* 1979). Briefly, these trials indicate that redheaded pine sawfly NPV can be successfully applied by aircraft, and that it is highly effective for control of this pest. A virus dosage of 5×10^9 polyhedral inclusion bodies (PIB)/ha gave excellent control when applied at a rate of 9.4 L/ha.

Until 1979, the redheaded pine sawfly NPV aerial spray tank mixes included an ultraviolet light protectant, first IMC 90-001, then Sandoz Shade®.[1] In the 1979 field trials, the efficacy of NPV formulated in 25% v/v animal feed grade molasses, 60 g/L Sandoz Shade and water was compared to NPV formulated in water only. The dosage (5×10^9 PIB/ha) remained the same for both treatments.

Although a virus dosage of 5×10^9 PIBs/ha emitted at 9.4 L/ha gave excellent control, ultra low volume (ULV) sprays promise a more economical and efficient method for treating insect-infested plantations. To test the ULV concept, the effectiveness of an aerial application of NPV at a dosage of 5×10^9 PIB/ha in emitted volumes of 2.35, 4.70 and 9.40 L/ha for control of redheaded pine sawfly was assessed in 1980. A study was also undertaken in 1980 to compare a purified virus suspension with the standard, lyophilized, NPV-infected larval material.

This report presents the results of the 1979 and 1980 field trials.

BIOLOGY AND ECOLOGY OF THE INSECT

The biology and ecology of redheaded pine sawfly have been extensively studied by Middleton (1921) and Benjamin (1955). In Canada, adult sawflies emerge from overwintered cocoons in the duff layer of the soil from mid-June to early July. The female usually lays 80-140 eggs ($\times 120 \pm 6.72$) (Griffiths 1960), and the egg incubation period is usually 3 to 4 weeks in southern Ontario and Quebec. The gregarious larvae feed on foliage of all ages, but prefer foliage from the previous year. Sawfly larvae become fully grown in 25 to 30 days from time of emergence. Sawfly larvae pass through 5 feeding instars and 1 non-feeding instar prior to spinning their cocoons. Prepupae drop to the ground where they spin a cocoon in the surface layer of the soil. Just prior to emergence, the prepupa transforms into a pupa and shortly thereafter develops into the adult stage. In Canada this species has only one generation per year, whereas in the southernmost parts of its range it may have as many as 5 generations per year.

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[1] The biological control division of International Minerals Corporation was taken over by Sandoz Wander Inc. and the two products, IMC 90-001 and Sandoz Shade are one and the same.

Red pine, *Pinus resinosa* Ait., is the principal species attacked in Canada, followed closely by Scots pine, *P. sylvestris* L., and jack pine, *P. banksiana* Lamb. Serious outbreaks and tree mortality were relatively uncommon until the establishment of pure pine plantations became a common practice about 50 years ago. The significant increase in the number of hard pine plantations over the last 20 years in Ontario and Quebec is believed to be largely responsible for the current outbreak and serious pest status of this sawfly.

MATERIALS AND METHODS

The Virus

The virus for these programs was propagated by treating and harvesting colonies of larvae in the field. When redheaded pine sawfly larvae reached the fourth-instar they were sprayed, using a backpack mistblower, with an aqueous virus suspension containing 10^6 PIB/mL. When the sawfly colonies appeared diseased and moribund, twigs with insects were clipped and placed in paper bags. The diseased larvae were removed from the foliage with fine forceps, frozen, lyophilized, and ground to a fine powder in a Waring blender. This unpurified virus material contains less than 0.1% polyhedral inclusion bodies by weight, with the remaining material being milled insect parts. Different batches vary in their PIB content, but it is usually in the range of 2×10^9 to 2×10^{10} PIB/g. The virus material was stored in this powder form at 4°C until needed.

The Study Area

In 1979, the study area was located in the Ontario Ministry of Natural Resources (OMNR) administrative district of Pembroke, except the untreated check which was located near Sheenboro, Quebec (Fig.1). In 1980, the trial was conducted in the OMNR district of Minden (Fig. 2).

Plots were selected on the basis of sawfly distribution and the level of infestation, as well as on aircraft operability. If the infestation was localized within a small portion of the plantation, or if the population density throughout the entire plantation was less than one sawfly colony per 5 trees, the plantation was regarded as being unsuitable for an aerial spray program. Some plantations were rejected because of adverse aerial spray conditions such as obstructions from large trees within or surrounding the plantation, or because of irregular topography. Those plantations which met the necessary criteria were selected as study plots. Unfortunately, replications of the treatments were not possible during either year since additional acceptable sites were not available.

All areas selected for the field trials were red pine plantations, which are described in Table 1.

Larval Development

Implementing control as soon as larvae hatch and begin to feed is necessary to minimize tree damage since severe defoliation and even tree death can occur in one year, especially when trees are less than 5 years old. Prompt action and optimal timing of the treatment is important because mortality due to viral infection is not immediate after ingestion of polyhedral inclusion bodies, and may take several days or even 2 or 3 weeks before its impact is complete. Therefore, to ensure optimally timed applications of virus, the treatment plots were monitored and the percentage egg hatch and the larval development recorded daily.

Formulation and Mixing Procedures

Suspensions of crude virus were used for all the treatments except Plot 4 (1980), where a purified suspension of PIB was used. A stock suspension of the crude virus was made in the laboratory by mixing the lyophilized, virus-infected, larval powder in water using a Waring blender. A quantity of this stock suspension was purified using differential centrifugation and retained for use in Plot 4. Just prior to

Table 1. Description of the study plots

Plot No.	Location	Plot size (ha)	Tree age (yr)	Mean tree height (m)	Mean number of colonies per tree	Mean number of larvae per colony
1 (1979)*	South Alona Twp. Renfrew County Ontario	5	8	2.0	7.7	46
2 (1979)	Ross Twp. Renfrew County Ontario	16	8	2.1	0.5	28
Check (1979)	Sheen Twp. Pontiac County Quebec	1	6	1.6	0.9	64
1 (1980)	Anson Twp. Ontario	7	9	2.9	0.5	151
2 (1980)	Minden Twp. Ontario	18	12	1.4	1.7	108
3 (1980)	Snowdon Twp. Ontario	4	12	2.3	2.3	198
4 (1980)	Stanhope Twp. Ontario	4	4	1.1	0.3	55
Check (1980)	Minden Twp. Ontario	25	15	7.0	0.6	86

* Year treated.

the aerial spray, the final aqueous formulations were prepared by adding the appropriate volume of the virus suspension to the tank mix ingredients. Rhodamine B dye was added at 0.04% v/v to the tank mix as a tracer for spray card analysis on all plots except Plot 1 (1979), the sunscreen and molasses tank mix being sufficiently dark to mark cards. The formulation for each treatment is given in Table 2.

Spray Application and Larval Development

Treatment, time of application, larval development and meteorological conditions at time of spray for each plot are given in Table 2. A Cessna 185E equipped with 4 Micronair® AU 3000 units was used for all applications. Meteorological conditions at the time of spray were recorded only in Plot 1 (1979) and in Plot 1 (1980). Ideally, meteorological conditions

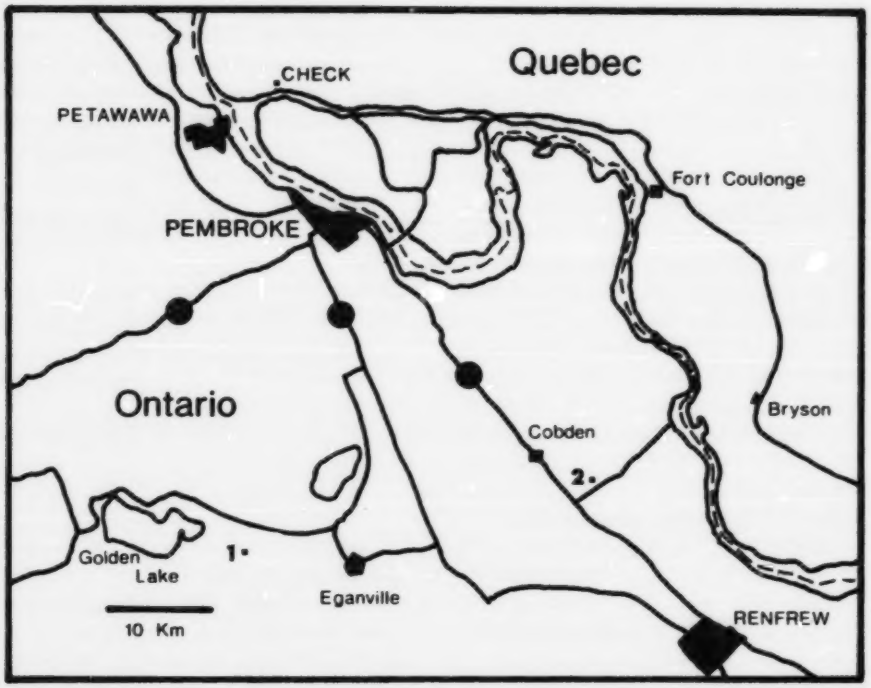


Figure 1. Locations of experimental plots in 1979.

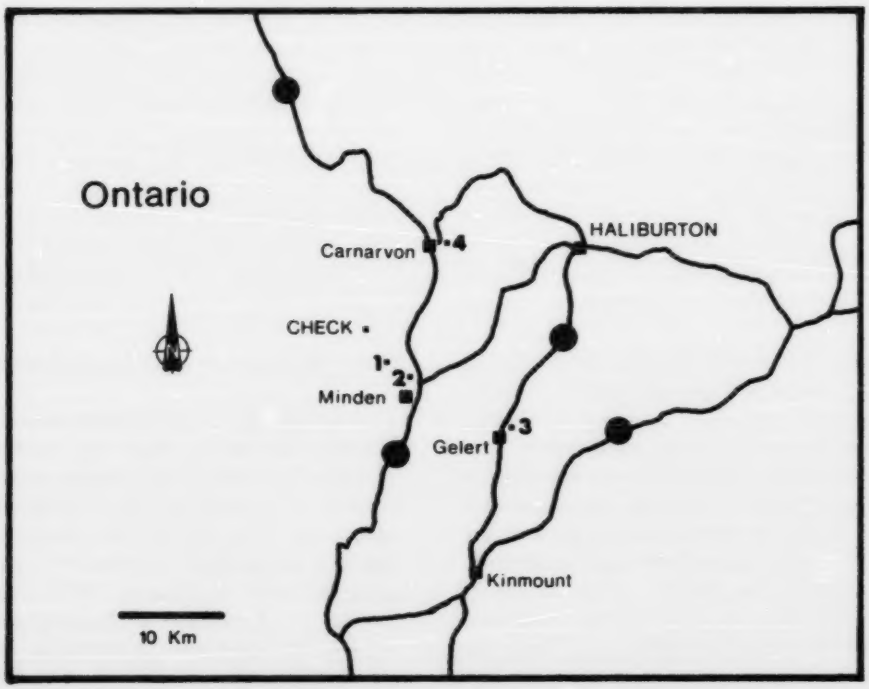


Figure 2. Location of experimental plots in 1980.

Table 2. Treatments, timing of application, larval development, and meteorological conditions at time of spray.

	Plot 1 1979	Plot 2 1979	Plot 1 1980	Plot 2 1980	Plot 3 1980	Plot 4 1980
Treatment	5×10^9 PIB/ha emitted in 9.40 L/ha formulated with 60 g/L Sandoz Shade® and 250 ml/L molasses	5×10^9 PIB/ha emitted in 9.40 L/ha unformulated	5×10^9 PIB/ha emitted in 2.35 L/ha unformulated	5×10^9 PIB/ha emitted in 4.70 L/ha unformulated	5×10^9 PIB/ha emitted in 9.40 L/ha unformulated	5×10^9 PIB/ha emitted in 9.40 L/ha unformulated purified virus
Time of application	July 10 0515-0520	July 10 0635-0645	July 14 0606-0614	July 14 0645-0705	July 14 0733-0741	July 14 0525-0540
Larval development at time of spray	45% L_1 55% L_2	49% L_1 51% L_2	66% L_1 34% L_2	58% L_1 42% L_2	77% L_1 23% L_2	70% L_1 30% L_2
Meteorological conditions at time of spray	No inversion[1] wind speed 0-2 kph R.H. 95%*[2] Temp. 15°C	Conditions similar to Plot 1 1979	Inversion +2°C mean wind speed 1 kph R.H. 100% Temp. 7°C	No inversion estimated wind speed 3-5 kph R.H. 98% Temp. 7°C	No inversion estimated wind speed 5-6 kph R.H. 88% Temp. 8°C	Inversion[3]+ estimated wind speed <1 kph R.H. 100% Temp. 6°C

[1] Visual observations of spray cloud suggest the presence of ground convection currents and thermal uplift.

[2] Relative humidity measured with a sling psychrometer.

[3] Since Plot 4 (1980) was sprayed before Plot 1 (1980) where a positive inversion was present, it was assumed that Plot 4 also had a positive inversion.

should be measured in each treatment plot at the time of the spray. It is desirable, however, that treatments being compared be applied within the shortest time-span possible to ensure uniformity in the development of the insects between the plots, and to reduce meteorological variations betweeen the treatments. Since it was impractical to move meteorological equipment to each plot within the time-span allotted, it was decided to place the tower in a plot which was considered to be the most suitable to provide the weather data. The wind speed and the air temperature at 3 and 6 m above the ground were recorded using a Heathkit Digital Weather computer ID-4001 30 min prior, during and 30 min after the spray application. Relative humidity was measured with a sling psychrometer.

Deposit Sampling

The spray deposit was sampled to determine the droplet size spectrum and the number of droplets/cm^2. The sampling device consisted of a Kromekote® card mounted on a 10 cm^2 aluminum backing. Immediately before spraying, the cards were placed at 15 m intervals across each plot at right angles to the flight lines. The cards were collected approximately 30 min after the application.

A microcard reader with calibrated grid screen was used to count the number of droplets/cm^2. To estimate the droplet density on the Kromekote cards, five 1 cm^2 sections were randomly chosen and the droplet stains were recorded. To determine the diameter of droplet stains, the droplets were projected onto the screen of the micro-card reader and the stain diameter was measured with a calibrated plastic stencil.

Assessment of Virus Efficacy

To determine the impact of the virus application, 100 trees with 1 colony/tree were selected and tagged in each spray plot and each check plot. Beginning 2 days post-spray, they were examined daily for symptoms of virus infection. The colonies were checked until the larvae died or until they pupated. Virus infection begins after the insect has consumed virus-contaminated foliage and eventually the mid-gut becomes milky white due to the large number of PIBs which form in these cells. Death follows soon after infection, and dead larvae adhere to the foliage by their prolegs. Microscopic examination of the guts from individual sawfly larvae which showed virus symptoms (white mid-guts or larvae hanging by their prolegs) confirmed that such larvae were heavily infected with NPV following an aerial spray with this virus (Kaupp and Cunningham 1977; Kaupp *et al.* 1978). No microscopic diagnosis was undertaken in 1979 and 1980 and the colonies exhibiting these symptoms were recorded as virus-infected.

The times taken by the virus to cause first mortality and to eventually decimate the colony were recorded. The developmental stage of the insects when these two events occurred was also noted. Similar observations were made on the untreated check plots and the percent mortality due to the spray treatment was calculated from the difference in survival between the treated and untreated plots.

RESULTS

Deposit Sampling

Droplet density (number of droplets/cm^2) and droplet stain size distribution are given in Table 3. The deposit on Plots 1 and 2 (1979)was considered excellent with 93 droplets/cm^2 on Plot 1 and 135 droplets/cm^2 on Plot 2. Nearly all the droplet stains measured on the cards were <150 µm. Droplet densities for the 1980 spray trials were also considered very satisfactory, with at least 90% of the droplet stains <150 µm.

Assessment of Virus Efficacy

1979 Trials. First signs of virus infection were noted 5 and 6 days post-spray, and by 23 days post-spray, all larvae were dead in Plots 1 and 2. The temporal increases in the number of colonies with first mortality and com-

plete mortality are shown in Figures 3 and 4. In both plots complete mortality occurred before larvae reached the fourth-instar in all marked colonies. No virus infection was noted in the untreated check plot; hence, virus mortality in the spray blocks was considered to be entirely due to the application.

1980 Trials. First mortality due to virus infection occurred in all 4 treated plots by 8 days post-spray. By 12 days post-spray, at least 50% of the colonies showed symptoms of virus infection and, at 10 days post-spray, all but 1 colony (Plot 3) showed these symptoms.

Complete mortality of one colony was observed in Plot 3 ten days post-spray. Two weeks after the spray, all 4 treated plots had at least 1 colony that was completely killed by virus, and by 3 weeks post-spray over 85% of the colonies examined were completely killed. Complete mortality due to virus infection occurred before larvae reached the fifth-instar with 33% dying in the fourth-instar and the remaining 67% in the third-instar. The temporal increases in first recorded mortality and complete mortality for the study plots are shown in Figures 5 and 6.

The experiment was terminated 31 days post-spray, by which time all the colonies in the spray plots had been killed by virus. During this time, none of the 100 colonies in the untreated check plot showed any signs of virus infection, nor had pupation commenced. Since there was 100% survival in the untreated plot, the differences in survival between the treated plots and the untreated check plot were considered to be entirely due to the spray application.

DISCUSSION AND CONCLUSIONS

There were no apparent differences in the efficacy of NPV in water alone and NPV formulated with 25% molasses and 60 g/L Sandoz Shade. It was expected that the addition of molasses would reduce the volatility of the spray droplets and therefore improve on-target droplet deposition, which in turn, might result in improved efficacy over the water-alone virus formulation. The card analysis revealed that Plot 1, water-alone formulation, received 135 droplets/cm^2 and that Plot 2, water, molasses and sunscreen formulation, received 93 droplets/cm^2

Table 3. Droplet density and size distribution recorded from spray cards analysed from plots sprayed in 1979 and 1980.

Plot No.	Average no. of droplet stains per cm^2	Percentage droplets in each stain size category			
		0-75 µm	76-150 µm	151-250 µm	251-350 µm
1(1979)	135	44.3	54.3	1.2	0.2
2(1979)	93	73.8	23.3	2.7	0.2
1(1980)	64	55.0	44.1	0.9	0.0
2(1980)	91	57.8	36.4	5.7	0.1
3(1980)	39	65.3	29.5	4.8	0.4
4(1980)	115	39.9	52.0	7.2	0.9

Fig. 3. 1979 temporal increase in number of sawfly colonies where 1st mortality due to virus infection was observed.

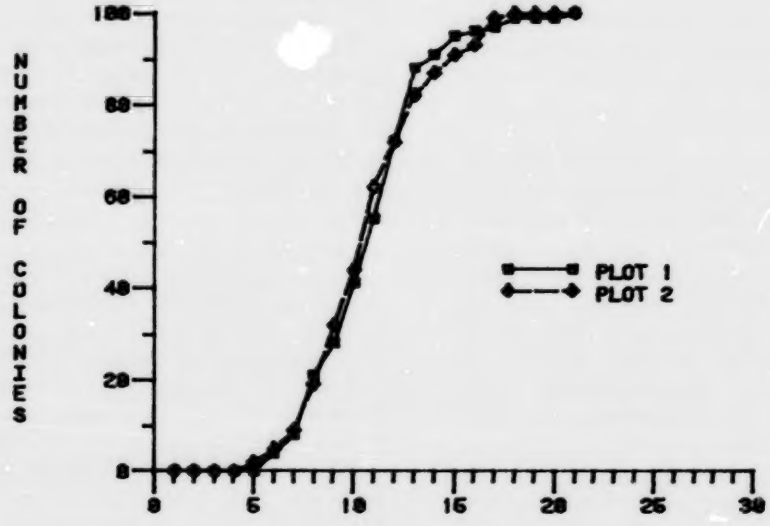


Fig. 4. 1979 temporal increase in number of sawfly colonies where complete mortality due to virus infection was observed.

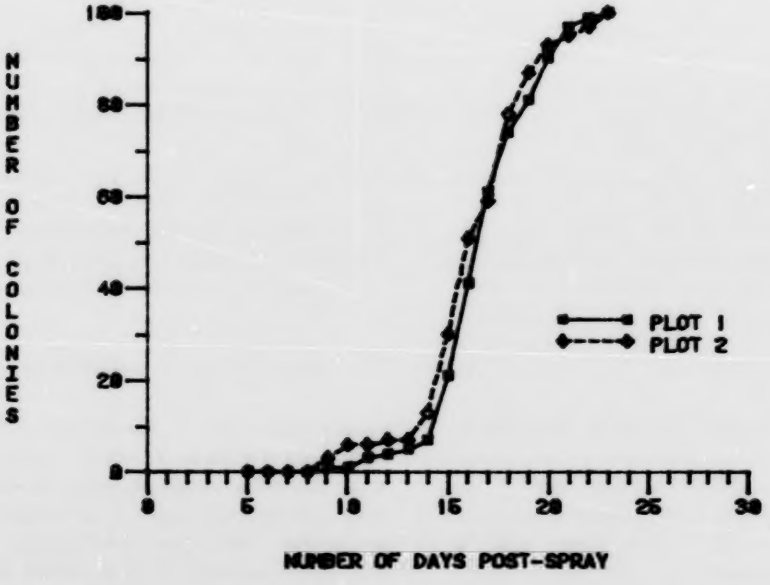


Fig. 5. 1980 temporal increase in number of sawfly colonies where 1st mortality due to virus infection was observed.

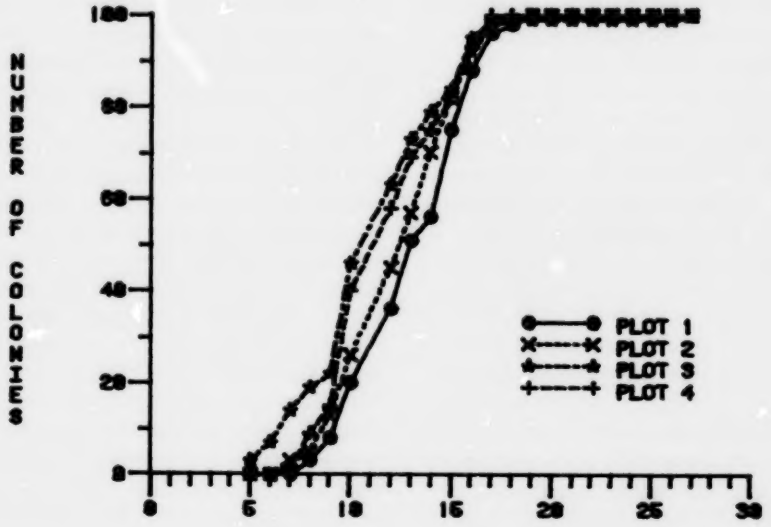
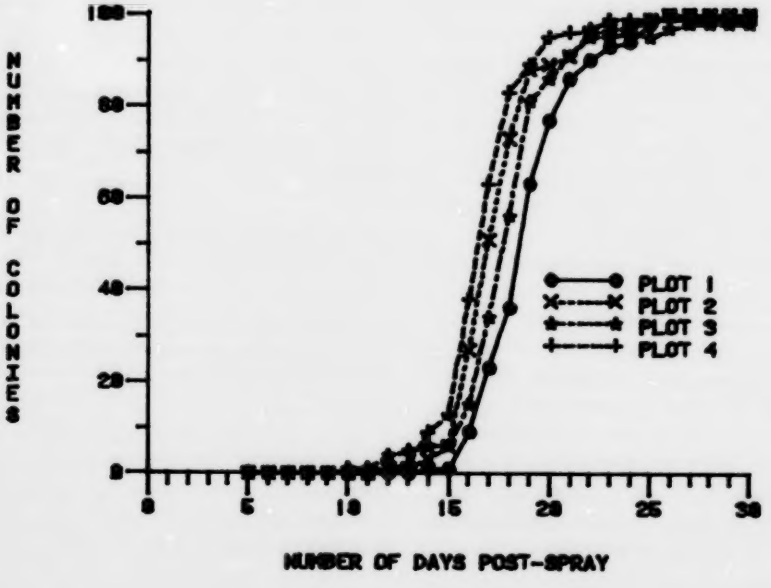


Fig. 6. 1980 temporal increase in number of sawfly colonies where complete mortality due to virus infection was observed.



which was the reverse of what one might expect. Generally, horizontal spray cards are poor collectors of spray deposit, especially for small droplets, and the analysis, therefore, should not be regarded as definitive.

As a measure of spray deposit, card analysis alone cannot explain the apparent similarity in efficacy between the two formulations. Among other things, wind speed, relative humidity, spray release height and topographical variability influence spray deposition. To determine unequivocally if the addition of molasses to the formulation improves virus efficacy, all other factors should be held reasonably constant, something that is difficult to do in aerial spray trials. The mortality pattern in the 1980 trials, with the water-alone formulation, was very similar to that of the 1979 trial, which used the molasses-based formulation. This lends some credence to the assumption that the addition of 25% molasses does not enhance efficacy for aerial sprays with this NPV. Furthermore, when one compares the mortality patterns in the 1977 and 1978 trials where the water-based formulation plus molasses was used, the results are similar.

Considering the mode of action of the virus and development of a virus epizootic, some of these similarities between treatments become apparent. Kaupp (1981) demonstrated that if one larva in a colony of *N. sertifer* (Geoff.), becomes infected, the rest of that colony will be infected after 4 days. Also, virus spreads from diseased to healthy colonies and complete spray coverage may not be necessary. In 1979, a "zebra stripe" application of redheaded pine sawfly NPV, where swaths were 100 m apart instead of the usual 30 m, demonstrated that complete coverage was not necessary to achieve control with this virus (Desaulniers and Cunningham, unpublished). While it is not disputed that molasses is a humectant, it is felt that considering the evidence accumulated to date on comparisons between formulated and unformulated tank mixes, and taking into consideration the behaviour of the virus in relation to its host, the addition of molasses to the tank mix is unnecessary.

Inactivation of PIB by ultraviolet (UV) light has been known for some time. Therefore, the addition of a sunscreen protectant such as Sandoz Shade should reduce UV inactivation and lead to improved efficacy because the PIB should remain viable for a longer time on the foliage. Again, judging from results obtained from the 1979 trial, it appears that the addition of Sandoz Shade does not improve the efficacy of an aerial application of this virus. That is not to say that this or other UV protectants will not be beneficial when used in conjunction with other viruses. Since mortality of the colony due to virus infection is not immediate, the forest manager and the research scientist must determine if the addition of a UV protectant is warranted, on the assumption that it would result in accelerated insect mortality because a larger number of viable polyhedra are ingested over the same time period than may occur without sunscreen. Certainly the addition of a UV protectant might result in a smaller quantity of virus being required. In making such a decision, one should consider the economics of adding a protectant and reducing dosage with that of increasing the dosage to compensate for UV inactivation without the addition of a UV protectant. Considering the relatively low production cost of this virus (about $2.50/ha on a non-profit basis for a dosage of 5×10^9 PIB), the expense of a UV protectant does not seem to be justifiable. Sandoz Shade is no longer available commercially and there are presently no UV screens marketed for use with viral pesticides.

Foliage consumption is most significant in the later instars. To reduce the damage impact, viruses should be applied as soon after sawfly eclosion as possible. Spraying at first-or second-instar is recommended and this practice has frequently resulted in significant foliage protection.

Varying the volume emitted per hectare between 2.35 and 9.40 L/ha appeared to have no bearing on the efficacy of the virus. An emitted volume of 2.35 L/ha gave a mortality

pattern very similar to that of 4.70 and 9.40 L/ha. It is suspected that within this range of application rates flooding of the Micronair AU 3000 units occurred. Flooding reduces optimum atomization of the spray resulting in a greater number of large droplets being produced. Himel (1969) advocated that maximum control of an insect pest with minimum use of a pesticide and minimum off-target drift can be achieved when droplets of optimum size are used. Since volume increases as a cube function of the spray droplet diameter, the amount of pesticide wasted rapidly increases when the droplet is above optimum size. Optimum droplet size will vary with different target surfaces, but in general, droplets less than 100 µm in diameter are most effectively collected by insects and foliage (Matthews 1977). Larger droplets (>100 µm) will generally be filtered out by the top canopy layer of the forest leaving the smaller droplets to penetrate the crop. Since it is known that there is a mechanical limitation to spray atomization when using Micronair AU 3000 units, it follows that there will be less penetration of the spray within the crop due to inadequate atomization at high volumes. The point at which spray coverage is inadequate due to poor atomization of the spray liquid was not demonstrated in this trial. Conversely, the minimum volume required to achieve adequate spray coverage was also not demonstrated. In many operational spray programs using chemical insecticides for control of spruce budworm, *Choristoneura fumiferana* (Clem.), the volume rate used is 1.5 L/ha. Further studies should be directed at determining the lowest effective application rate for redheaded pine sawfly NPV, perhaps using 1.5 L/ha as one of the rates to be tested.

In comparing the results of the trial using purified virus with those of the trials using crude virus preparations, it appears that there is no difference in efficacy between the two. It was thought that the purified virus may be less effective than the crude material containing insect protein which may protect the virus from inactivation by sunlight. The results of this trial did not confirm this assumption. An advantage of purifying virus suspensions is that they are easier to standardize and suspend in water than the crude material. There is a loss of polyhedra during the purification stage and this, along with the subsequent increase in cost, makes it less attractive as a pest control product than the crude material. However, while these results suggest there are no advantages to using purified virus over the crude material in terms of efficacy, the purified suspensions might be more acceptable environmentally. Crude extracts (impure preparations) may contain other viruses, rickettsia, bacteria and protozoa. Extensive safety testing of the crude virus suspension has been undertaken at Ontario Veterinary College, University of Guelph where the crude preparation of virus was found to present no hazard to mammals (Forsberg, Valli and Dwyer, unpublished), birds (Valli and Claxton, unpublished) and fish and *Daphnia pulex* (Hicks *et al.* 1981). It is recommended that prior to field use each batch of virus stock be bioassayed and subjected to quality control procedures. If harmful organisms are found, that particular batch should be purified or rejected.

In summary, it is quite clear that redheaded pine sawfly NPV is a very efficacious biocontrol agent. Excellent results, judged by complete mortality due to virus infection of the observed colonies, were obtained using an aqueous tank mix with no additives, applied at a dosage of 5×10^9 PIB/ha, emitted at a rate of 2.35 L/ha. We recommend, based on the results of these and previous field trials, that the lyophilized, virus-infected, finely ground larval powder be applied in water alone at a dosage of 5×10^9 PIB/ha. An application rate of 2.35 L/ha is suggested if the Micronair AU 3000 rotary atomizer units are used.

In addition to the need for replication of some of these trials, two important questions remain unanswered, (1) what is the lowest effective dosage required for an aerial application and, (2) what is the lowest effective application rate required? While it may appear that these questions are academic considering the low cost and effectiveness of the recommended dosage, it must be remembered that production of virus stocks is labour intensive and the use of an aircraft is expensive. Therefore,

exploring methods of reducing dosages and application rates can be justified from an economic viewpoint.

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